10.1111/j.1469-0691.2008.02142.x

Dynamics of natural infection by *Anaplasma phagocytophilum* in a dairy cattle herd in Brittany, France

E. Laloy[1], *E. Petit*[1], *H.-J. Boulouis*[1], *C. Gandoin*[1], *C. Bouillin*[1], *G. Gounot*[2], *S. Bonnet*[1] *and R. Maillard*[1]

[1]UMR BIPAR, Maisons-Alfort and [2]Clinique vétérinaire, Fougères, France

CONTEXT AND OBJECTIVE

Various mammals host *Anaplasma phagocytophilum*, formerly *Ehrlichia phagocytophila*. This agent is responsible for tick-borne fever (TBF) in domestic ruminants. The hard tick *Ixodes ricinus* is the main TBF vector in Europe.

Seasonal variations in seroprevalence to *A. phagocytophilum* in cattle has already been studied in a Swiss area where TBF was endemic by Pusterla *et al*. [1]. Cattle grazed on a pasture in a subalpine-to-alpine region from May to the end of September.

The aim of our study was to describe the dynamics of natural infection in cattle when grazing on tick-infested pastures in an oceanic climate.

MATERIALS AND METHODS

In Brittany (France), four groups of five cows or heifers were randomly selected within a dairy herd known to have been infected by *A. phagocytophilum* since 2005. One-year-old heifers were *A. phagocytophilum*-naive. They were turned out for the first time on 13 April 2007, whereas the others began to graze on 28 February 2007. The older animals had previously been exposed to tick bite.

In order to determine the moment of infection, blood and serum samples were collected every 15 days from 1 April to 15 November 2007, and the cattle were subjected to clinical examination.

An indirect immunofluorescence assay was performed to titrate anti-*A. phagocytophilum* IgG in bovine sera. Diluted serum was screened with a cut-off titre of 1 : 100.

Changes in white and red blood cell counts were evaluated. Blood samples were also used for DNA extraction. Genomic amplification of 16S rRNA, *groEl*, *gltA*, *msp2* and *msp4* genes of *A. phagocytophilum* [2–4] and sequencing were performed to search for evidence of bacteraemia before and during the first seroconversion, for each animal.

Corresponding author and reprint requests: R. Maillard, UMR BIPAR, 23 av. Général De Gaulle, Maisons-Alfort cedex, France
E-mail: rmaillard@vet-alfort.fr

No conflicts of interest declared.

RESULTS

Nineteen out of 20 animals were seropositive at least once during the grazing period. Before pasturing, the five naive heifers were seronegative; seroconversion occurred for two of them 2 weeks after turnout. Seroprevalence increased from 25% in April to 75% in June. On 10 September, only 50% were seropositive. Seroprevalence then increased again, and reached 80% on 1 November; it then decreased, and was 65% 2 weeks later.

Titres increased and decreased in parallel with the seroprevalence. The highest titres (3200) were more frequent in spring than in autumn. Follow-up of antibody titres (Fig. 1) often showed several peaks by animal. In spring, peaks were seen in May and June. In autumn, antibody peaks were more frequent in the second part of October. However, peaks were not synchronous among cattle.

Four animals were PCR-positive for 16S rDNA, *groEl* and *msp4* on 15 April (a naive heifer and a cow) and 1 May (two heifers). None of the four animals showed PCR-positivity 15 days before or after this unique seropositivity, suggesting detection duration of less than 15 days. All of them

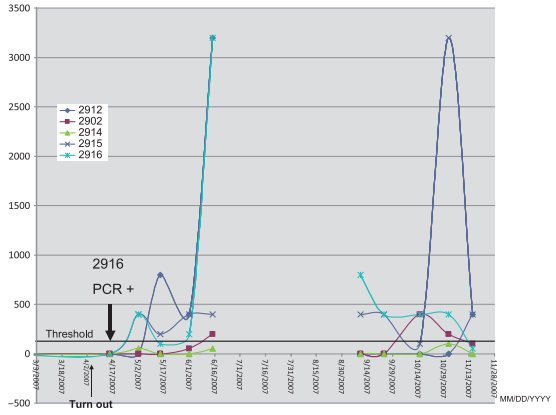

Fig. 1. Follow-up of anti-*Anaplasma* phagocytophilum antibody titres in sera of 1-year-old heifers during the 2007 pasture season. Each number in the top left corner corresponds to one heifer.

were seropositive at the same time, except for the naive heifer, in which seroconversion occurred 2 weeks later. This unique seropositivity, suggesting detection duration of less than 15 days.

In spite of their positivity, cattle showed neither clinical signs of TBF nor haematological changes after turnout.

DISCUSSION

This study confirmed seasonal variations in seroprevalence and serum titres of IgG to *A. phagocytophilum*, as highlighted by Pusterla *et al.* [1]. In that study, the number of seropositive Swiss cattle increased continuously throughout the pasture season, with a maximum in September. We demonstrated two peaks in seroprevalence: the number of seropositive Breton cattle increased in spring, decreased during summer and increased again in autumn, and then dropped after the middle of November. Variations in vector activity could explain these fluctuations, given that high precipitation and low temperatures in Brittany that year (http://www.meteofrance.com/FR/climat/dpt_tempsdumois.jsp?LIEUID=DEPT35) were likely to inhibit *I. ricinus* activity in August and at the end of November, respectively.

We detected PCR positivity to *A. phagocytophilum* in four animals, but none of them was apparently ill. In the Swiss cattle, *A. phagocytophilum* was detected in 12 cows by PCR. Among them, the two that had not been on the pastures with endemicity before had clinical signs of TBF. Therefore, lack of TBF signs in infected cattle could be due to immunity acquired in the previous pasture season [1]. However, in the herd that we studied, one PCR-positive heifer had not been on the pasture before. The infective dose inoculated by the vector may have been too low to trigger TBF. Inconsistent expression of the disease might also be due to genetic variants of *A. phagocytophilum* which could differ in pathogenicity [5].

CONCLUSIONS AND PROSPECTS

Serological and PCR results gave evidence of *A. phagocytophilum* infection within the herd. Infection occurred during spring and autumn, the active seasons for *I. ricinus* in an oceanic climate.

Our study revealed a discrepancy between serological results and clinical or haematological examination. Diagnosis by molecular biology was only possible for a few days.

The study will be pursued during the 2008 pasture season on the same cows to compare serological and clinical reactions depending on the immune status.

ACKNOWLEDGEMENTS

Our thanks go to M. Berrich, P. Betton and V. de Moustier for their technical assistance.

REFERENCES

1. Pusterla N, Berger Pusterla J, Braun U, Lutz H. Serological, hematologic, and PCR studies of cattle in an area of Switzerland in which tick-borne fever (caused by *Ehrlichia phagocytophila*) is endemic. *Clin Diagn Lab Immunol*, 1998; **5:** 325–327.
2. Massung RF, Slater K, Owens JH *et al.* Nested PCR assay for detection of granulocytic *Ehrlichiae*. *J Clin Microbiol*, 1998; **36:** 1090–1095.
3. Sumner JW, Nicholson WL, Massung RF. PCR amplification and comparison of nucleotide sequence from the *groESL* heat shock operon of *Ehrlichia* species. *J Clin Microbiol*, 1997; **35:** 2087–2092.
4. De La Fuente J, Massung RF, Wong SJ *et al.* Sequence analysis of the *msp4* gene of *Anaplasma phagocytophilum* strains. *J Clin Microbiol*, 2005; **43:** 1309–1317.
5. Stuen S, Solli Oppergaard A, Bergstrom K, Moum T. *Anaplasma phagocytophilum* infection in north Norway—The first laboratory confirmed case. *Acta Vet Scand*, 2005; **46:** 167–171.